

The Effect of Body Size on Male-Male Combat in the Parasitoid Wasp *Melittobia digitata* Dahms (Hymenoptera: Eulophidae)

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Abstract.—The parasitic wasp *Melittobia digitata* Dahms (Hymenoptera: Eulophidae) is a gregarious external parasitoid of various insects, primarily solitary wasps and bees. Males of *M. digitata* commonly engage in fierce, often fatal, fights with other males. The mandibles are the main weapons used, and injuries inflicted vary greatly in severity from loss of appendages to death. We investigated the effect of size differences between winning, losing, and non-fighting males and whether body size was related to being a fighter or non-fighter. The head width and tibia length of fighting and non-fighting males were measured. Winning males (21 of 29 pairs) were found to be larger than losing males, and fighting males (winners and losers combined) were found to be larger than non-fighting males (8 pairs). Mandible lengths of a subset of all males (15 fighters, 12 non-fighters) were subsequently measured; only fighter's left mandible length was significantly correlated with head width. The possibility that two behavioral forms (fighters and non-fighters) exist is considered but will require further experiments to resolve.

Ritualized fighting over food, territory, mates and other resources occurs between males in the majority of animal species. Many studies have emphasized the restrained nature of male fights by showing that the majority of fights end peacefully with neither combatant being injured, even in species where the males possess large and dangerous weapons (Maynard Smith and Price 1973; Maynard Smith 1982). In addition, fighting males often display striking dimorphisms such as those found in certain beetles (Forsyth and Alcock 1990; Zeh et al. 1992; Goldsmith and Alcock 1993) and mites (Saito 1990, 1995). Instances of fatal fighting have been observed in some animal species. Where they do occur, fatal fights usually involve opportunities to mate, and they are limited to species where males have limited opportunities to mate (Enquist and Leimar 1990). Fatal fighting has been well documented in many fig wasps (Hamilton 1979; Murray 1987; Bean and Cook 2001). These wasps spend their entire life within

the swollen inflorescences of various fig species, and fights occur between well-armed flightless males that pursue newly emerged females. The majority of males do not emerge from the fig in which they are born though emergence is more common in some species (Bean and Cook 2001). Males have also been shown to have a strong attraction to the fig in which they were reared (Frank 1985), which makes dispersal unlikely. Since potential mating opportunities are limited, fights between males are fierce and result in many fatalities.

Melittobia digitata Dahms (Hymenoptera: Eulophidae) is a gregarious external parasitoid of many different insects, but its principal hosts are solitary wasps and bees. In the southeastern United States, *M. digitata* is most commonly found attacking the mud dauber wasp, *Trypoxylon politum* Say (Hymenoptera: Sphecidae). A female *Melittobia* enters a *Trypoxylon* cocoon before it is sealed and waits until the host transforms into a prepupa before ovipos-

iting hundreds of eggs directly onto the prepupa's cuticle (Dahms 1984; González and Terán 2001). Upon hatching, the gregariously developing *Melittobia* larvae consume the host, complete their development and begin to emerge in approximately 20 days, depending on temperature. The average brood size for *M. digitata* resulting from one female foundress is 522 young. Males and females emerge together, but the sex ratio is extremely female biased—about 98% female (J.M. González, personal communication). Females characteristically mate once, usually soon after emergence, and one male may mate with numerous females in his lifetime. Mated females then chew their way out of the host's cell and disperse to search for new hosts (Dahms 1984).

Sexual dimorphism is extreme in *Melittobia*. Adult males possess vestigial eyes, short non-functional wings, enlarged antennal scapes, and mandibles with well-developed teeth. The blind males wander freely inside the host's cocoon until they encounter a female or another male. Encounters with females instigate courting and mating behaviors, and virgin females often gather in groups around males to await mating (González et al. 1985; Consóli et al. 2002). Encounters with other males quickly escalate into a grappling contest where the males interlock their legs and struggle briefly with each other. Following these bouts, the males will either separate or attempt to use their mandibles to tear at the body of the opponent. These fights often lead to loss of appendages and death in one or both fighters (Dahms 1984). Inside naturally parasitized mud dauber cocoons, one routinely finds the remains of several males, many dismembered. In most laboratory cultures of *M. digitata*, males grapple and fight with little provocation, and these contests frequently end with the death of one or both combatants. In other cultures, we have found many males alive with no injuries and no evidence of fighting. Long term

culturing in the laboratory does not alter *Melittobia* behavior (Assem and Jachmann 1999), so intense fighting is not likely to be an artifact of mass rearing.

The occurrence of fatal fighting in both fig wasps and *Melittobia* is unexpected because males are normally fighting their brothers. Hamilton (1979) suggested that fighting behavior would not exist where a male's rival has a high chance of being a brother. Recent work with fig wasps, however, found no relationship between relatedness of males and fighting behavior. It was found instead that the level of fatal fighting was negatively correlated with future mating opportunities (West et al. 2001). A similar situation exists in *Melittobia* where males have little chance of future mating opportunities since they are not likely to disperse.

The objective of this study was to determine if size differences exist in *M. digitata* between winners and losers of fights and between fighting and non-fighting males. We hypothesized that winners would be larger than losers and that fighters would be larger than non-fighters.

MATERIALS AND METHODS

Melittobia digitata cultures were reared in an incubator at 25°C on *T. politum* prepupae in small plastic boxes (5cm × 2.5cm × 1.8cm) with tightly fitting lids. Males were removed from cultures as pupae and isolated in CarolinaTM clear Deep Well Projection Slides (25 mm diameter, 2 mm deep). This isolation ensured that a male's age and prior mating and fighting experience could be controlled. No data were recorded on male emergence time relative to other males from a particular culture nor from which culture a given male emerged. Thus, males used in the experiments can be regarded as arbitrarily selected from among a range of males available.

When the males isolated in the depression slides emerged, the date of their emergence was recorded. Eighty-seven

Table 1. Morphometric measurements of *M. digitata* males.

	Total number	Mean tibia length*	Mean head width*
Winners	21	0.25 ± 0.03	0.35 ± 0.04
Losers	21	0.23 ± 0.03	0.34 ± 0.04
Fighters	42	0.24 ± 0.03	0.35 ± 0.04
Non-Fighters	16	0.20 ± 0.03	0.29 ± 0.03

* Values in mm ± Standard Deviation.

males were kept isolated and observed daily, and their date of death was recorded. These males served as controls for the following experiment.

Twenty-nine newly emerged male pairs were formed in the depression slides by opening the two individual slides and using a paintbrush to move one of the males into the other male's slide. Because of the difficulty of marking individuals, it was not possible to track which male was resident versus intruder in the pairings. Once each pair had been formed, the slides were not opened again until after both males had died. Each pair was observed daily, and the date of each male's death was recorded.

After death, each male was examined, and any obvious injury (e.g., loss of appendages, body wounds) was recorded. The head and right front tibia of each male were then mounted temporarily in glycerol on standard glass microscope slides and measured under 50× magnification using an ocular micrometer. Both males of one pair were mounted on the same slide so that winners and losers could be associated. To avoid crushing the specimens, pieces of 6lb. test nylon fishing line were placed around them to elevate the cover slip.

To test whether head width is correlated with mandible length, 15 fighter heads and 12 non-fighter heads were arbitrarily selected, and their mandibles were dissected. Both left and right mandibles were mounted on microscope slides and their maximum length was measured.

Sign tests were used to analyze winner

versus loser data so that both males of one fighting pair could be compared against each other. Mann-Whitney U tests were used to analyze fighter versus non-fighter data. Spearman R Correlations were used for all correlations. A *P* value of 0.05 was taken as the critical value for establishing significance. Analyses were done using STATISTICA 6 © StatSoft, Inc.

RESULTS

In 21 of the pairs, one male killed the other in a fight. These males were termed fighters. In eight of the pairs, the males were never observed to come into contact with each other, and after death neither male was found to have lost appendages or incurred wounds to the body. We concluded in these cases that no fighting had occurred, and these males were recorded as non-fighters.

Table 1 shows the measurement data for all males.

Winners had significantly longer tibia ($Z = 2.29$; $P = 0.022$), but head widths of winners and losers did not differ significantly ($Z = 1.21$; $P = 0.228$). The tibia length of all fighters (winners and losers combined) was significantly longer than the non-fighters' tibia length ($U = 134.0$; $Z = 3.58$; $P < 0.001$), and fighters' heads were significantly wider than non-fighters' ($U = 88.5$; $Z = 4.34$; $P < 0.001$). Head width and tibia length for all males (fighter and non-fighter) were significantly correlated ($\rho = 0.665$; $P < 0.001$).

The only significant correlation between head width and mandible length was found for fighters' left mandibles ($\rho =$

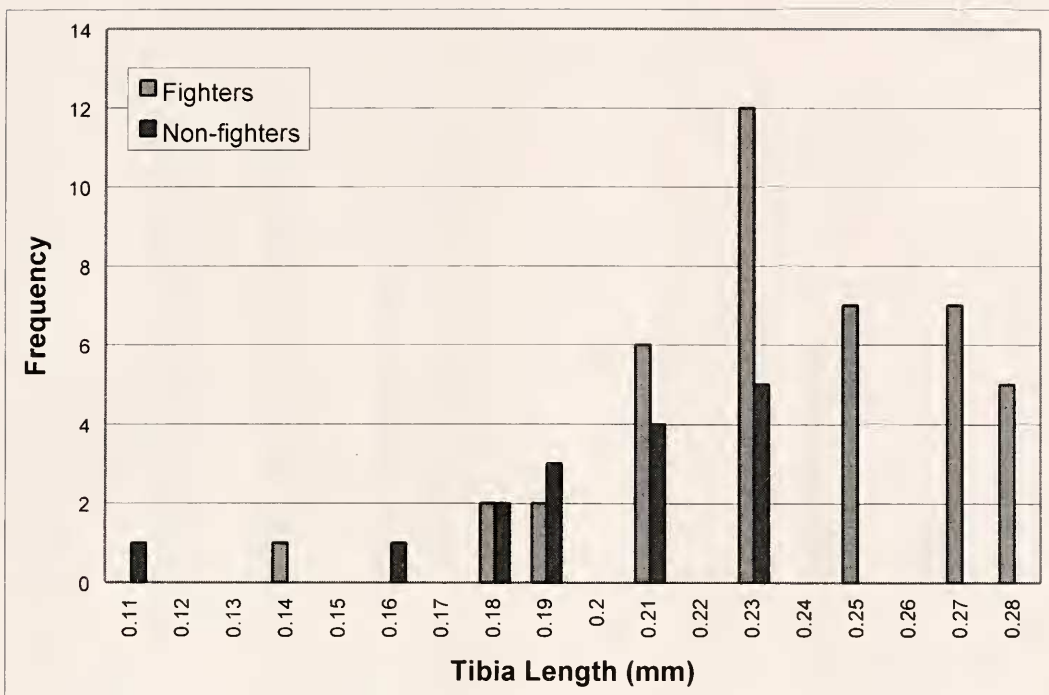


Fig. 1. Frequency distribution for tibia length comparing all fighters and non-fighters ($n = 58$).

0.692; $P = 0.004$). Fighters' right mandibles were not significantly correlated with head width ($\rho = 0.351$; $P = 0.200$). Non-fighters' left mandibles were not significantly correlated with head width ($\rho = 0.507$; $P = 0.092$), and non-fighters' right mandibles were not significantly correlated with head width ($\rho = 0.452$; $P = 0.140$).

Differences in size between fighters and non-fighters and the obvious behavioral differences suggested the possibility that the *Melittobia* male population could be dimorphic. To explore this possibility, frequency distributions of the measurements of tibia length and head width were prepared (Figs. 1 and 2). If a dimorphism exists, a bimodal curve is expected. The graph of tibia length frequency reveals only one peak for both fighters and non-fighters, and this peak occurs at 0.23 mm. The graph of head width frequencies suggests the presence of two peaks—one for non-fighters at 0.28 mm and one for fighters at 0.37 mm. There is, however, consid-

erable overlap, and values for fighters and non-fighters occur at both ends of the scale.

DISCUSSION

We predicted that winners would be the larger males. The results revealed that winners were larger than losers based on their tibia length measurements, although their head widths did not differ. The correlation between head width and tibia length suggests that relative size of either is likely to be a good predictor of overall body size, which in turn is related to fighting success. Mandible size has been often discussed in relation to fighting fig wasps (Bean and Cook 2001). The frequent asymmetry between right and left mandible lengths in our study was unexpected, and may be interesting to pursue.

The existence of non-fighter males, in which paired males never engaged in a fight even though they were isolated together for their whole life, raises the pos-

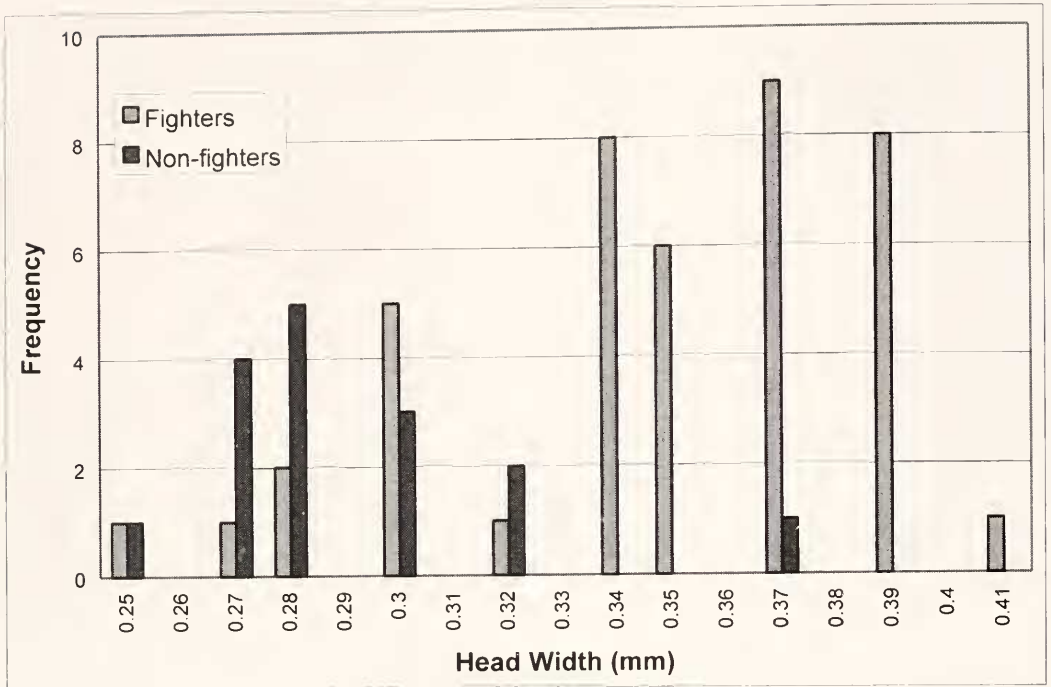


Fig. 2. Frequency distribution for head width comparing all fighters and non-fighters ($n = 58$).

sibility that males exist in two behavioral morphs. Freeman and Ittyeipe (1982) described two morphologically distinct male morphs in *Melittobia hawaiiensis* Perkins (= *M. australica* Girault): a larger morph with ocelli and a smaller morph without ocelli. However, we found that all of our *M. digitata* males, large and small, had fully pigmented ocelli. The frequency graphs of head width and tibia length (Figs. 1 and 2), while showing a trend towards a bimodal distribution for each trait, also reveal that a wide range of sizes exists in both fighting and non-fighting males. Overall, most of the larger males became fighters while most of the smaller males never engaged in fights, but there were obvious exceptions. Perhaps fighting and non-fighting are conditional rather than fixed traits. Alternatively, there could be culture or lineage specific effects on the likelihood of a male becoming a fighter or non-fighter. These questions will require further investigation.

Abe et al. (2003) found that when an emerged male and a pupal male were placed together, the already emerged male usually killed the pupal male at or immediately after eclosion, but they did not record sizes of any of the males in their experiment. We have noted that the first males to emerge are generally larger most likely due to better food quality and quantity, and small males emerge later in the culture's life, when the host is covered with developing pupae (unpublished data). Males of different fig wasp species are known to exist in a wide variety of body shapes, each with a different fighting propensity, and each is adapted to courting females in a different way (Murray 1990). If fighting behavior in *Melittobia* is linked to size, then perhaps a similar situation occurs with small, non-fighting males being better adapted to maneuvering amongst the developing pupae in the tightly packed confines of a *Trypoxylon* cocoon. They could avoid the stress of fight-

ing by staying hidden, but they could still court females. Repeating these experiments using males from one culture and tracking relative emergence times could help to determine if males that emerge early tend to be larger and become fighters and males that emerge late tend to be smaller and become non-fighters.

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